Non-Invasive Hemoglobin Monitoring during Hemorrhage and Hypovolemic Shock

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ABSTRACT

Background: *Serial blood draws for the assessment of a trauma patient's hemoglobin (sHgb) and hematocrit (sHct) is standard practice. In the event of multiple casualties this process can be time consuming and lead to the inefficient use of valuable resources. A device that would allow for continuous real-time, non-invasive monitoring of hemoglobin and tissue perfusion would not only improve the utilization of scarce and valuable resources but would also improve triage efforts.* ***Purpose:*** *We developed a device utilizing the technology of Diffuse Optical Spectroscopy (DOS) to obtain non-invasive measurements of tissue hemoglobin concentration (THC) and oxygen consumption in an animal model of hypovolemic shock induced by successive blood withdrawals. Measured DOS results were compared against invasive systemic physiological measurements to demonstrate that DOS provides a reliable non-invasive measurement of tissue THC, and also quantifies various degrees of hemorrhage induced systemic hypovolemia and subsequent tissue perfusion decreases.* ***Methods:*** *Intubated New Zealand White rabbits (N=16) were hemorrhaged via a femoral arterial line every 10 minutes until a 20% blood loss (10-15 cc/kg) was achieved to attain hypovolemia. A DOS probe was placed on the inner thigh to measure muscle concentrations of oxygenated-Hgb (Hb-O_2) and deoxygenated-Hgb (Hb-R), during bloodletting. THC and tissue hemoglobin saturation (S_TO_2) were calculated using oxygenated and deoxygenated hemoglobin concentrations. DOS-measured values were compared against traditional invasive measurements, systemic hemoglobin (sHgb), arterial oxygen saturation (S_AO_2), and venous oxygen saturation (S_VO_2) drawn from arterial and central venous blood. Systemic blood pressure (mAP), heart rate (HR) and S_AO_2, were monitored throughout the entire experiment.* ***Results:*** *DOS and traditional invasive measurements versus blood loss were closely correlated (R=0.98 and R=0.97, respectively) showing a decline in both. S_TO_2 and Hb-O_2 followed similar trends with hemorrhage whereas an increase in Hb-R was observed.* ***Conclusion:*** *DOS provides a potential platform for reliable non-invasive measurements of tissue oxygenated and deoxygenated hemoglobin and may accurately reflect the degree of systemic hypovolemia and compromised tissue perfusion.*

INTRODUCTION

Hemorrhage remains a leading cause of death in combat and major trauma [1]. In multiple traumas, rapid assessment of victims that are critically volume depleted is necessary to reduce morbidity and mortality associated with hypoperfusion and gauge resuscitation. However, during the acute hemorrhage, systemic

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hematocrit (sHct) and hemoglobin (sHgb), may be artificially normal or increased due to the inherent lag time in the body's fluid shifts[2]. Therefore, observation in peripheral perfusion may be a more representative indicator of volume status. A means for a quick and portable non-invasive assessment of tissue hemoglobin concentration (THC) and perfusion would increase efficiency in diagnosing patients in greatest need for volume replacement and may aid in the assessment of those patients undergoing resuscitation. In the hospital setting, evaluation of the critically ill patient often consists of systemic hemodynamic monitoring by invasive central venous access and by serial blood draws. Therefore, having the ability to assess tissue hemoglobin and perfusion parameters non-invasively in real time may reduce the cost and complications associated with invasive monitoring techniques.

To address these concerns, tissue oxygen hemoglobin saturation monitoring with near infrared spectroscopy (NIRS) has been proposed as a possible alternative to invasive monitoring [3-10]. These NIRS devices exploit blood chromophore properties of light absorption at characteristic frequencies. Accordingly, the amount of light absorbed is directly proportional to the chromophore concentration. These instruments, however, are limited by their ability to measure only light absorption and do not account for light scatter that occurs in complex tissues. While NIRS devices are able to monitor relative changes in tissue chromophore concentration, this results in significant limitations, with the inability to accurately differentiate absolute concentration of oxygenated tissue hemoglobin ($Hb\text{-}O_2$) from deoxygenated tissue hemoglobin (Hb-R).

Diffuse optical spectroscopy (DOS) is a novel technique that is able to simultaneously measure both light absorption as well as light scatter in turbid media and tissue [11-15]. Instrumentation based on this theory is not bound by the restrictions seen in NIRS, and as a result has the potential to accurately measure absolute tissue chromophore amounts; especially those of considerable importance, $Hb\text{-}O_2$ and Hb-R. Using a prototype instrument developed in our laboratory [11, 12, 16, 17], we were able to demonstrate that DOS-derived physiologic hemoglobin properties correlated with invasive measurements of cardiac output (CO), mean pulmonary artery pressure (mPAP), mean systemic arterial pressure (mAP), and arterial oxygen saturation (S_AO_2) [17]. In this report, to address how DOS reflects changes of sHgb during blood volume depletion, we compared hemoglobin obtained from a complete blood count (CBC) drawn invasively to non-invasive tissue hemoglobin DOS measurements (THC = $Hb\text{-}O_2$ + Hb-R) with emphasis in delineating $Hb\text{-}O_2$ from Hb-R.

MATERIAL AND METHODS

This protocol was approved the University of California, Irvine Animal Research Committee, protocol No. 2000-2218.

Anesthesia and Intubation. Male New Zealand White rabbits (N=16) (Myrtle Rabbitry Inc., Thompson Station, TN) weighing 4.0 $\pm$ 0.4 kg were anesthetized with a ratio of Ketamine HCI (100mg/ml) (Ketaject, Phoenix Pharmaceutical Inc., St. Hoseph, MI):Xylazine (20mg/ml)(Anased, Lloyed Laboratories, Shenandoa, IA) at a dose of 0.75 cc/kg IM. After the IM injection a 22-24 gauge 1 inch catheter was placed in animal's marginal ear vein to administer IV anesthesia and secured with 1 inch standard porous adhesive tape. Maintenance anesthetic was dosed at 0.3cc of 1:1 mixture of Ketamine:Xylazine IV (Ketamine 100 mg/ml:Xylazine 20 mg/ml) as needed. Depth of anesthesia was monitored according to established guidelines [18]. Animals were intubated with a 3.0 endotrachael tube and mechanically ventilated (Harvard apparatus dual phase control respirator: South Natick, MA) at respiration rate of 32/min and a tidal volume of 50cc and FiO_2 of 100%. Pulse oximetry was accomplished with a probe placed on the forlimb to measure S_AO_2 (Biox 3700 Pulse Oximeter, Ohmeda, Boulder, CO) and compared to arterial blood gas measurements.

Cardiac Output and Pulmonary Artery Pressure. After adequate anesthesia a median sternotomy was performed to expose the heart. A calibrated flow transducer (T106 small animal flow meter, Transonic System, Inc, Ithaca, NY) was placed around the ascending aorta to determine cardiac output (CO). The mean CO was determined from a 10 second sample. Pulmonary artery pressures were obtained by placement of an 18-gauge catheter in the pulmonary artery and connected to a calibrated pressure transducer (TSD104A transducer and MP100 WSW System, Biopac Systems, Inc, Santa Barbara) and collected digitally. Mean, systolic, and diastolic pressures were determined from 5-10 second tracings.

Blood Gas Analysis and Complete Blood Count. A right femoral arterial line was placed for arterial blood draws and systemic pressures. After all blood draws lines were flushed with less than 0.5 cc of heparin (Elkins-Sinn, Inc., Cherry Hill, NJ) to prevent line thrombus occlusion. Arterial blood samples were measured with a blood gas analyzer (IRMA Series 2000 Blood Analysis System, Diametrics Medical Inc., St Paul, MN). Mixed venous blood samples were drawn from the pulmonary artery.

Complete blood counts (CBC) were obtained from collected samples of venous blood and sent to an outside facility (Antech Diagnostics, Irvine, CA) for measurments.

Non-Invasive measurements (Diffuse Optical Spectroscopy). Detailed analysis of DOS has been described previously in detail [11, 12, 16]. Briefly, a multi-wavelength, frequency domain instrument (FDPM) was combined with a steady state near infrared (NIR) spectrometer for the non-invasive *in vivo* measurement of tissue chromophore concentration. Broadband DOS employs six laser diodes (661, 681, 783, 823, 850, and 910 nm) and a fiber-coupled avalanche photo diode (APD) detector (Hamamatsu high-speed APD module C5658). The APD detects the intensity-modulated diffuse reflectance signal at modulation frequencies between 50 to 550 MHz after propagating through the tissue. The absorption and reduced scattering coefficients are measured directly at each of the six laser diode wavelengths using the frequency-dependent phase and amplitude data [11-13, 16]. The reduced scattering coefficient is calculated throughout the NIR by fitting a power-law to these six reduced scattering coefficients [19-21]. The steady-state acquisition is a broadband reflectance measurement from 600 nm to 1000 nm that follows the FD measurements using a tungsten-halogen light source (FiberLite lamp) and a miniature spectrometer (Ocean Optics USB2000). The intensity of the steady-state (SS) reflectance measurements are calibrated to the FD values of absorption and scattering to establish the *absolute* reflectance intensity. The absolute steady-state reflectance spectra are then analyzed to calculate μ_a spectra. Finally, the tissue concentrations of Hb-O_2, Hb-R, and H_2O are calculated by a linear least squares fit of the wavelength-dependent extinction coefficient spectra of each chromophore.

Experimental Model. After completion of the sternotomy, baseline measurements of the above mentioned variables were obtained, and non-invasive assessment of Hb-O_2 and Hb-R was completed. The first hemorrhage was accomplished by withdrawing blood via a femoral arterial line (15cc). The blood drawing and measurement process was repeated every 20 minutes until a 20% blood loss (15 cc/kg) was achieved to attain hypovolemia. An entire experiment lasted for approximately 60 minutes. At completion of the experiment each animal was euthanized with an intravenous injection of Eutha-6 (1.0 -2.0 cc) through the marginal ear vein catheter according to animal laboratory guidelines (Institutional Laboratory Animal Care and Use Committee, University of California, Irvine ARC protocol No. 2000-2218).

RESULTS

Diffuse optical spectroscopy versus cardiac output and systemic mean arterial pressure. DOS measurements of THC and tissue oxygen saturation (S_TO_2 = [Hb-O_2/THC]*100%) demonstrated similar trends with systemic

arterial pressure and cardiac output (Figs 1 and 3). All parameters displayed a decreasing trend with successive blood withdrawal.

Diffuse optical spectroscopy versus systemic hemoglobin values. Both measurements of DOS THC and systemic hemoglobin (sHgb) displayed a downward trend with blood volume withdraw (Fig 2). DOS demonstrated a greater percent change when compared to systemic values. Significant differences compared to baseline ($p<0.05$) were noted in DOS THC and not sHgb at the initial blood draw. DOS THC and sHgb versus blood loss were closely correlated ($R=0.98$ and $R=0.97$, respectively).

Diffuse optical spectroscopy tissue oxygen saturation versus systemic arterial and venous oxygen saturation. Arterial oxygen saturation (S_AO_2) was maintained above 97% throughout the experiment. Systemic venous oxygen saturation (S_VO_2), however, decreased during blood volume withdrawal (Fig 3). Similarly, a decline of about 20% in DOS tissue oxygen saturation (S_TO_2) was observed. At the end of the hemorrhage period S_TO_2 appeared to be in equilibrium equivalent to S_VO_2 measured directly.

Diffuse optical spectroscopy tissue oxygenated and deoxygenated hemoglobin. When delineating tissue Hb-O_2 and Hb-R components from the combined THC, both Hb-O_2 and Hb-R showed a decline in an absolute amount during hemorrhage (Fig 4 and 5, respectively). This, however, was primarily due to the drop in the THC. When normalizing for this decrease in THC by dividing these parameters by the total tissue hemoglobin it was observed that the relative oxygenated hemoglobin (Hb-O_2/THC) displayed a significant drop (Fig 4). In contrast, an increase in the relative deoxygentated hemoglobin (Hb-R/THC) was observed (Fig 5). When taking a ratio of the absolute concentration of Hb-O_2 and Hb-R a decline of about 33% from baseline was noted (Fig 6).

DISCUSSION

These results demonstrate that DOS can non-invasively quantify Hb-O_2 and Hb-R. DOS measured decreases in THC, which paralleled sHgb, during hemorrhage. The high sensitivity of DOS is realized by greater percent changes in DOS-measured tissue hemoglobin concentration compared to changes in systemic invasively measured hemoglobin concentrations, which take time to equilibrate.

Previous NIRS studies have been applied in the use of monitoring tissue oxygen saturation [3-7]. These devices, however, are limited because tissue optical properties change under varying degrees of hypovolemia and, as a result, confound observations that are associated with the baseline measurements. While all NIRS devices can measure tissue oxygen saturation, many lack the ability to measure absolute concentrations of oxygenated and deoxygenated hemoglobin in tissue. This limitation arises from an inability to measure tissue light scattering, which is unfortunate because scattering is the dominant effect in NIR light transport [22]. To compensate for this, NIRS often uses calibration curves or average path length calculations derived from healthy subjects. These corrections can provide reliable measurements for hemodynamically stable patients. However, as stated above, since both tissue scattering and absorption change during volume depletion, these results become unreliable when acute systemic changes occur. In addition, photon path lengths display a high degree of intra-subject variation, which complicates absolute comparisons in both individuals and across populations [23]. DOS compensates for this by measuring both tissue absorption and scattering properties directly, and can therefore measure absolute tissue deoxygenated and oxygenated hemoglobin concentrations without the need to generate a calibration curve for each series of observations.

This added functionality serves well in assessing acute hemorrhage. Often systemic hemoglobin measurements do not reflect volume loss until compensation by the intracellular and interstitial fluid

compartment occurs [2]. Furthermore, vasoconstriction mechanisms by skin and muscle microvasculature compensate for hypovolemia by shunting blood centrally, and as a result, further decrease peripheral tissue hemoglobin. DOS provides a unique advantage by detecting these decreases in tissue hemoglobin sooner than that which would be observed by systemic hemoglobin measurements (Fig 2). Therefore, DOS may detect these peripheral changes initiated by hypovolemia before systemic signs are present and may provide further insight in regard to the patient's perfusion state.

Although volume depletion may be observed early with DOS, this does not imply the body is in oxygen debt. Previous studies have shown that the peripheral tissue is able to adjust metabolic demand to the available oxygen supply [24]. In the initial acute hemorrhage, compensation mechanisms allow for a continual oxygen supply for the vital organs. During more advance stages of acute blood loss, however, the body may exhaust its oxygen reserve and compensating mechanisms, resulting in a decrease in S_vO_2 (Fig 3), and produce an oxygen debt. Non-invasive measurements of tissue hemoglobin oxygen saturation in conjunction with systemic S_vO_2 values may elucidate this critical point. Since pulse oximetry relies mainly on arterial oxygen saturation and is mainly dependent on lung function and not the body's metabolic state, non-invasive measurements of S_TO_2 may also provide a significant compliment to these traditional measurements especially in environments where blood gas or central venous monitoring may not be available.

A critical advantage DOS has over previously described NIRS devices is the ability to measure absolute concentrations of Hb-O_2 and Hb-R. Out in the field, the initial assessment of a trauma victim is limited to available monitoring devices. As a result it is difficult to assess the degree of hypovolemia or more importantly, hypoperfusion. NIRS devices are limited in these situations because of the need to start with a baseline measurement and compare future measurements with these over a given time period or perturb tissue using phylesmographic analysis. Although these devices may be useful in the assessment of the resuscitation period, they lack the ability to assess the patient's initial perfusion state. DOS measurements provide the capability of giving an initial assessment to the perfusion state of the patient, by providing Hb-O_2 and Hb-R that is derived from absolute hemoglobin concentrations. For example, decreased ratios of Hb-O_2 to Hb-R suggest oxygen debt whereas increasing ratios are more suggestive of an oxygen reserve (Fig 6). Applying this data with the vital signs and clinical observation one may be able to provide a rapid assessment of the patients perfusion state, and depending on the available resources, initiate the resuscitation effort, or as in the case of multiple traumas rapidly move to assess the next injured party.

A significant amount of resources are needed for assessment of volume depletion in the critically ill patient, often consisting of surveillance serial blood draws. This is not only time consuming but occurs at a substantial price. A non-invasive, continuous real time assessment would be a more affordable and efficient way to monitor signs of hypovolemia and of possible hypoperfusion. DOS has the potential to offer these capabilities at the initial exam and over a continuous time period. If proven efficacious in the critical trauma setting, the eventual goal of the device would be a hand held portable system that would allow for transfer of data from medics in the field to physicians at a base if patients were not accessible to immediate health care. This device would not only free up valuable resources that are needed for mass causalities but could provide potential information and instruction from distant bases to assess those who are in most urgent need of care.

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REFERENCES

1. Dubick, M.A. and J.L. Atkins, *Small-volume fluid resuscitation for the far-forward combat environment: current concepts.* J Trauma, 2003. **54**(5 Suppl): p. S43-5.
2. Guyton, A., *Human Physiology and Mechanisms of Disease*. 6 ed. 1996.
3. Beilman, G.J., et al., *Near-infrared spectroscopy measurement of regional tissue oxyhemoglobin saturation during hemorrhagic shock.* Shock, 1999. **12**(3): p. 196-200.
4. Crookes, B.A., et al., *Noninvasive muscle oxygenation to guide fluid resuscitation after traumatic shock.* Surgery, 2004. **135**(6): p. 662-70.
5. Chaisson, N.F., et al., *Near-infrared spectroscopy-guided closed-loop resuscitation of hemorrhage.* J Trauma, 2003. **54**(5 Suppl): p. S183-92.
6. Torella, F., et al., *Monitoring blood loss with near infrared spectroscopy.* Comp Biochem Physiol A Mol Integr Physiol, 2002. **132**(1): p. 199-203.
7. Buchner, K., et al., *Near-infrared spectroscopy--not useful to monitor cerebral oxygenation after severe brain injury.* Zentralbl Neurochir, 2000. **61**(2): p. 69-73.
8. Boushel, R. and C.A. Piantadosi, *Near-infrared spectroscopy for monitoring muscle oxygenation.* Acta Physiol Scand, 2000. **168**(4): p. 615-22.
9. Lovell, A.T., et al., *Continuous measurement of cerebral oxygenation by near infrared spectroscopy during induction of anesthesia.* Anesth Analg, 1999. **88**(3): p. 554-8.
10. Shadid, M., et al., *Near infrared spectroscopy-measured changes in cerebral blood volume and cytochrome aa3 in newborn lambs exposed to hypoxia and hypercapnia, and ischemia: a comparison with changes in brain perfusion and O2 metabolism.* Early Hum Dev, 1999. **55**(2): p. 169-82.
11. Jakubowski, D.J., *Development of Broadband Quantitative Tissue Optical Spectroscopy for the Non-Invasive Characterization of Breast Disease*, in *Beckman Laser Institute*. 2002, University of California, Irvine: Irvine.
12. Tromberg, B.J., et al., *Non-invasive In Vivo Characterization of Breast Tumors Using Photon Migration Spectroscopy.* Neoplasia, 2000. **2**(1-2): p. 26-40.
13. Pham, T.H., et al., *Broad bandwidth frequency domain instrument for quantitative tissue optical spectroscopy.* Rev. Sci. Instrum., 2000. **71**: p. 2500-2513.
14. Fantini, S., et al., *Frequency-domain multichannel optical detector for noninvasive tissue spectroscopy and oximetry.* Appl. Opt., 1995. **36**: p. 10-20.
15. Pogue, B.W. and M.S. Patterson, *Frequency-domain optical absorption spectroscopy of finite tissue volumes using diffusion theory.* Phys. Med. Biol., 1994. **39**: p. 1157-1180.
16. Bevilacqua, F., et al., *Broadband absorption spectroscopy in turbid media by combined frequency-domain and steady-state methods.* Appl. Opt., 2000. **39**(34): p. 6498-6507.
17. Pham, T.H., et al., *Noninvasive monitoring of hemodynamic stress using quantitative near-infrared frequency-domain photon migration spectroscopy.* Journal of Biomedical Optics, 2002. **7**(1): p. 34-44.
18. Chen, J.C., et al., *Diffusing capacity limitations of the extent of lung volume reduction surgery in an animal model of emphysema.* J Thorac Cardiovasc Surg, 1999. **117**(4): p. 728-35.
19. Graaff, R., et al., *Reduced light-scattering properties for mixtures of spherical particles: a simple approximation derived from Mie calculation.* Appl. Opt., 1992. **31**: p. 1370-1376.
20. Schmitt, J.M. and G. Kumar, *Optical scattering properties of soft tissue: a discrete particle model.* Appl. Opt., 1998. **37**: p. 2788-2797.
21. Mourant, J.R., et al., *Predictions and measurements of scattering and absorption over broad wavelength ranges in tissue phantoms.* Appl. Opt., 1997. **36**: p. 949-957.
22. Wilson, B.C., et al., *Time-dependent optical spectroscopy and imaging for biomedical applications.* Proceedings of the IEEE, 1992. **80**(6): p. 918-930.

23. Quaresima, V., S.J. Matcher, and M. Ferrari, *Identification and quantification of intrinsic optical contrast for near-infrared mammography.* Photochem. Photobiol., 1998. **67**(1): p. 4-14.
24. Wilson, M., D.P. Davis, and R. Coimbra, *Diagnosis and monitoring of hemorrhagic shock during the initial resuscitation of multiple trauma patients: a review.* J Emerg Med, 2003. **24**(4): p. 413-22.

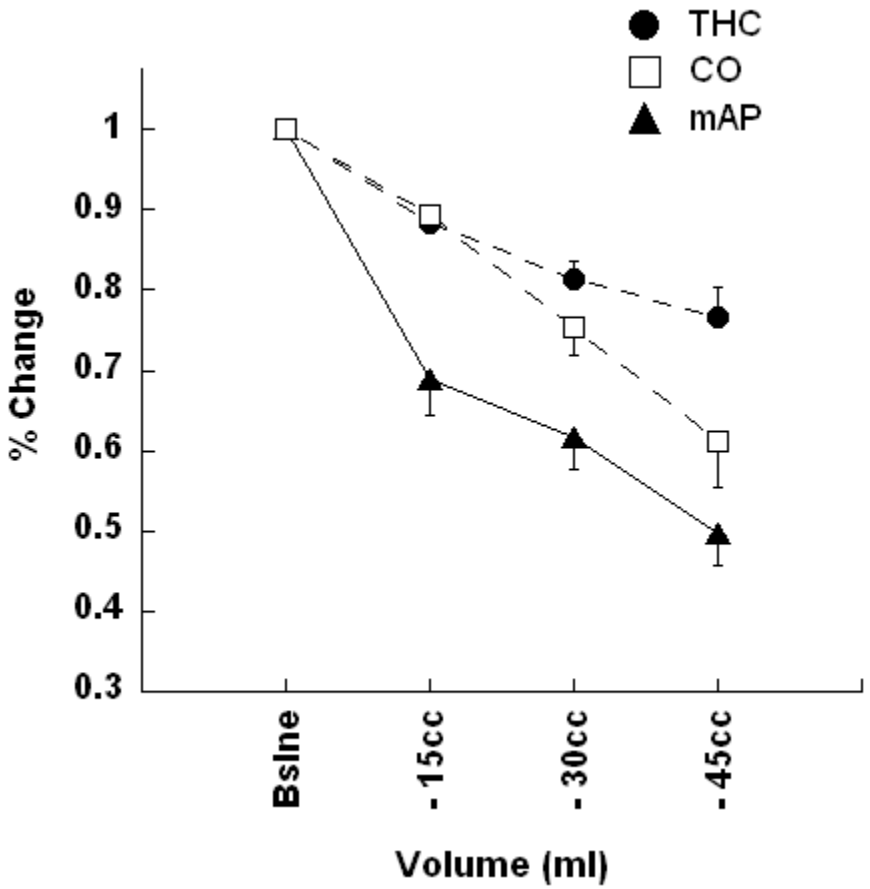

Figure 1*:* Total tissue hemoglobin concentration (THC) and systemic parameters versus volume loss. Cardiac output (CO) and mean arterial pressure (mAP) declined with hemorrhage when compared to baseline measurements. THC followed a similar trend.

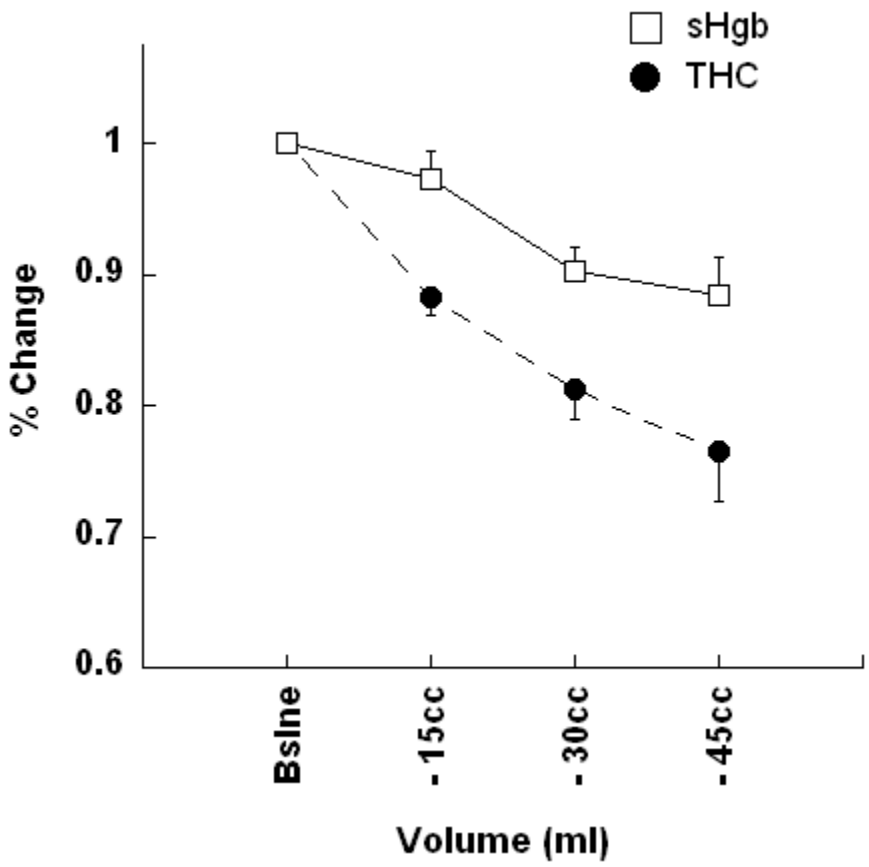

Figure 2: Tissue hemoglobin concentration (THC) and systemic hemoglobin (sHgb) versus blood volume loss. Both sHgb and THC demonstrated a drop with during progressive hypovolemia. A significant ($p<0.05$) reduction in THC during the initial blood draw was observed when compared to baseline measurements. This was not apparent with sHgb, although by the second blood draw both had significantly decreased.

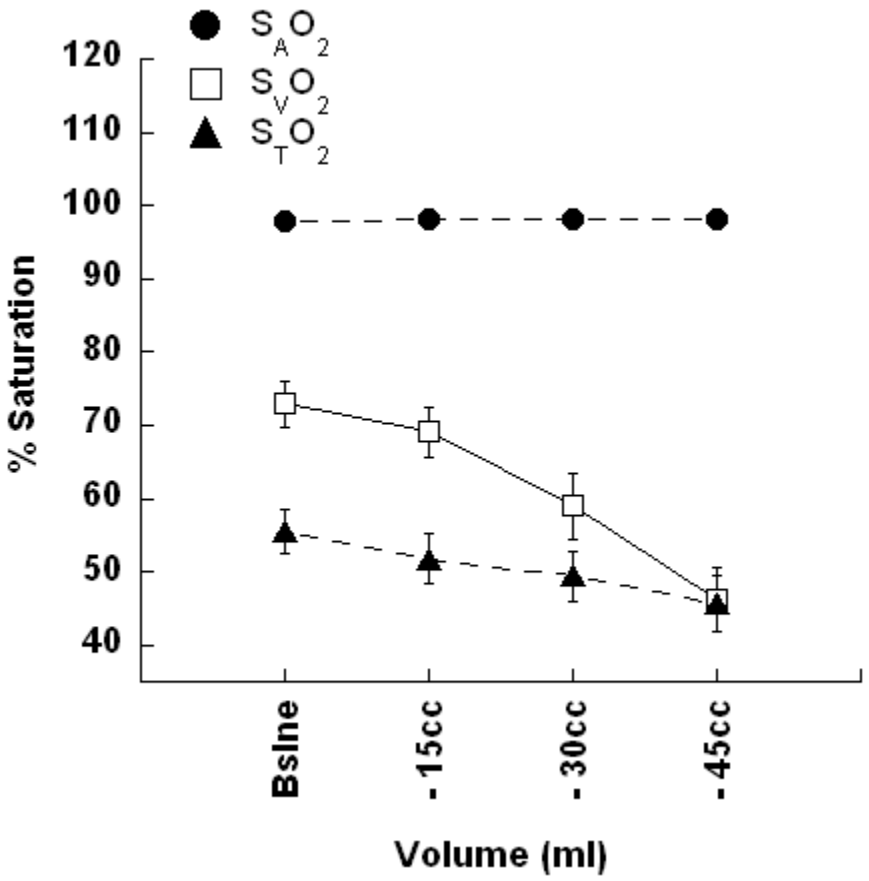

Figure 3: Arterial (S_AO_2), venous (S_VO_2), and tissue oxygen (S_TO_2) saturation versus hemorrhage. S_AO_2 was maintained constant while a decline in S_VO_2 occurred with blood loss. S_TO_2 obtained by DOS showed a 33% reduction when compared to baseline.

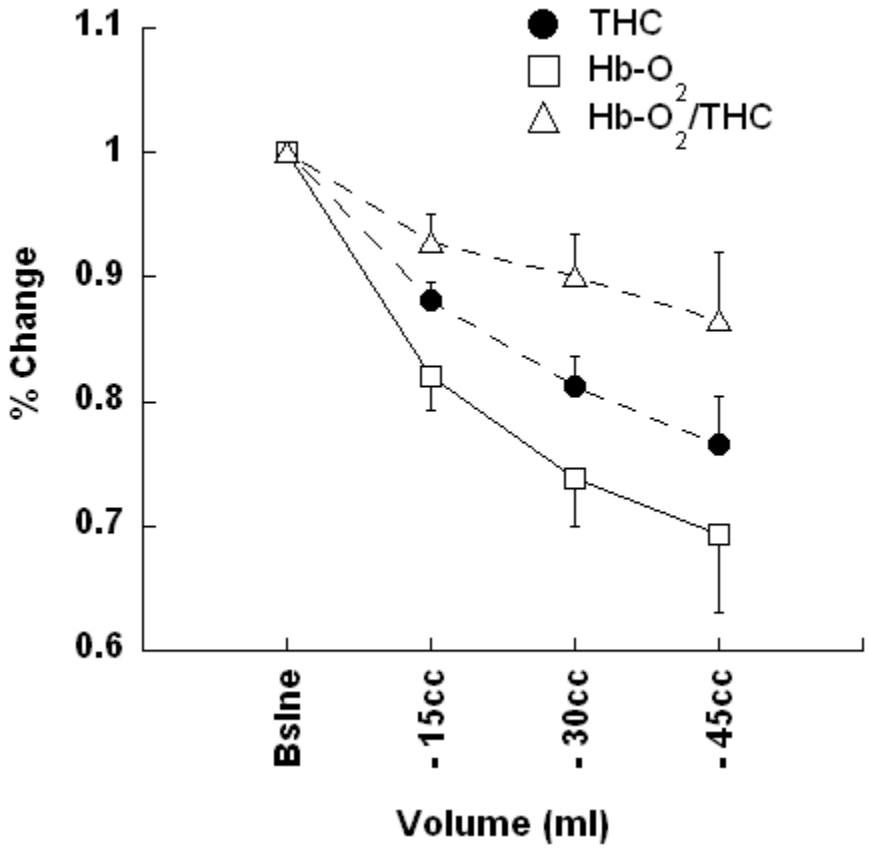

Figure 4: Non-invasive measurements of tissue oxygenated hemoglobin ($Hb\text{-}O_2$). A reduction in both THC and $Hb\text{-}O_2$ hemoglobin occurred with hemorrhage. When normalizing for the systemic drop (Hb-O2/THC) a notable decline was still present.

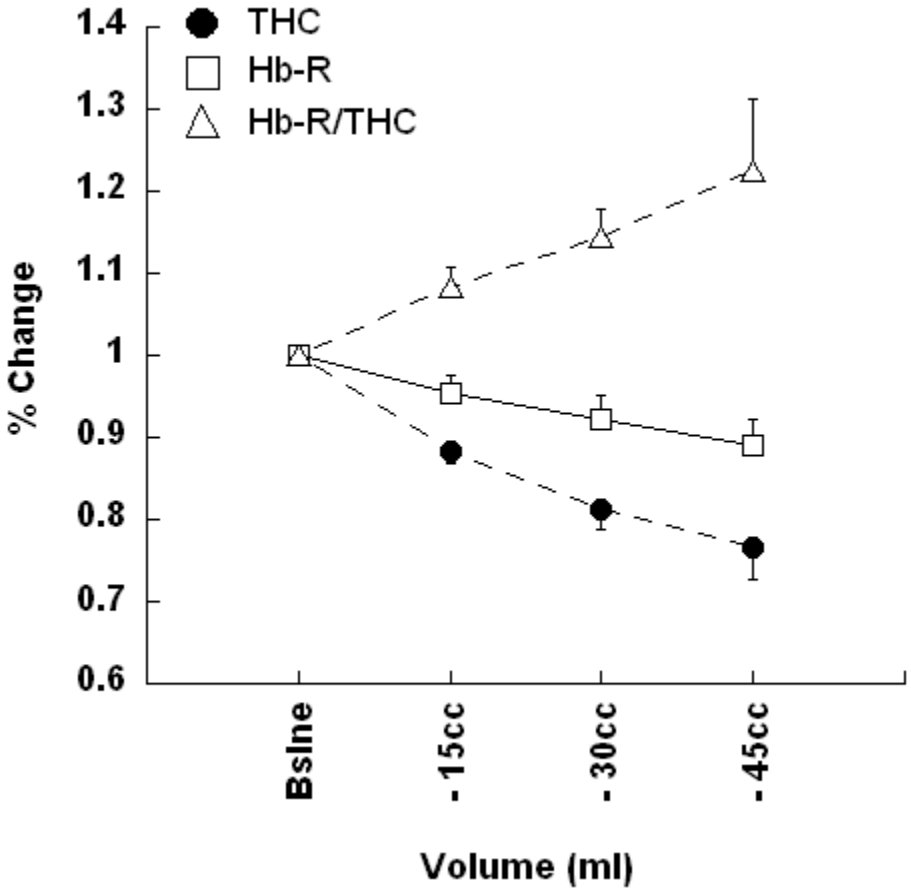

Figure 5: Non-invasive measurements of tissue deoxygenated hemoglobin (Hb-R). A drop in both THC and Hb-R occurred with blood loss. However, when normalizing for the systemic drop (Hb-R/THC) a relative increase of Hb-R was observed.

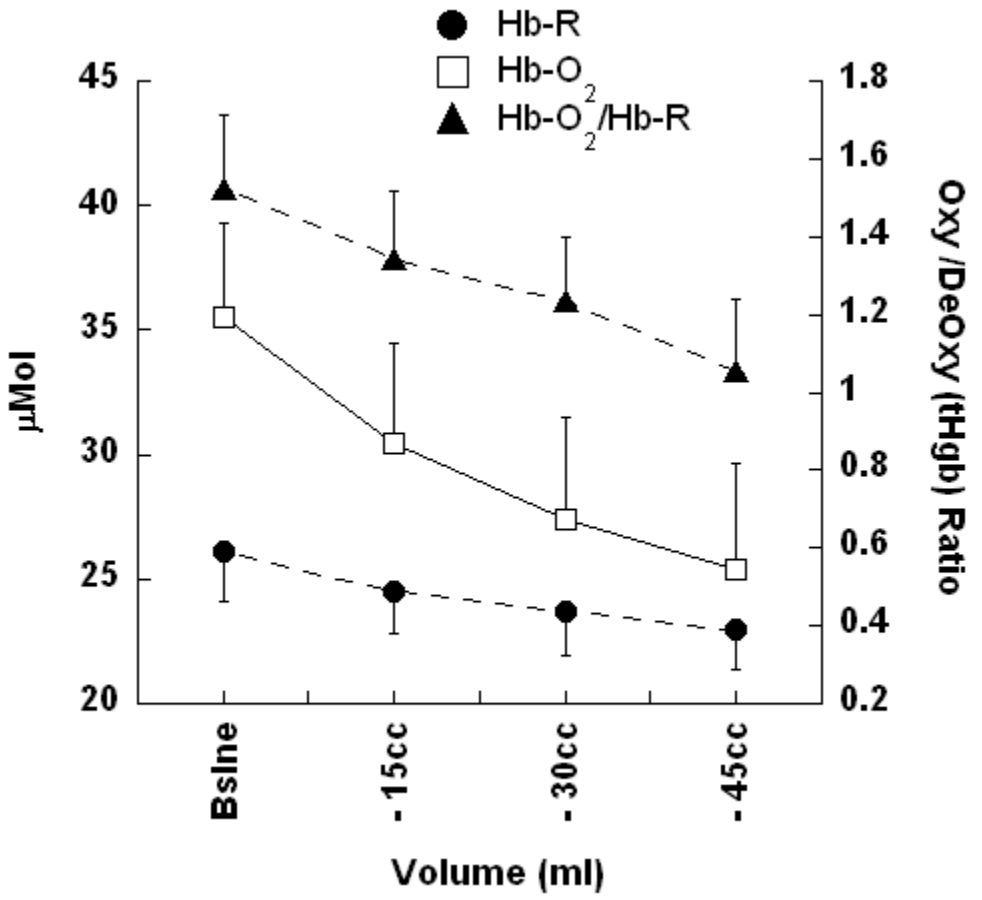

Figure 6: Absolute concentrations of tissue oxygenated (Hb-O_2) and deoxygenated (Hb-R) hemoglobin. Hb-O_2 decreased relative to Hb-R of about 33% from baseline.